A single nucleotide polymorphism in the *MicroRNA-146a* gene is associated with diabetic nephropathy and sight-threatening diabetic retinopathy in Caucasian patients.

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Abstract:

Aims: This study aimed to investigate whether the single nucleotide polymorphism (SNP) rs2910164, residing within micro-RNA146a (miR-146a) is associated with diabetic microvascular complications diabetic nephropathy (DN), proliferative diabetic retinopathy (PDR) or diabetic macular edema (DME) in either Caucasian patients with type 1 (T1DM) or type 2 (T2DM) diabetes mellitus.

Methods: Caucasian patients with T1DM (n=733) or T2DM (n=2215) were recruited from ophthalmology, renal and endocrine clinics in Australia and the United Kingdom. Patients with T2DM were required to have diabetes mellitus (DM) for at least five years and be on treatment with oral hypoglycemic drugs or insulin. 890 participants had DN (168 with T1DM and 722 with T2DM), 731 had PDR (251 with T1DM and 480 with T2DM) and 1026 had DME (170 with T1DM and 856 with T2DM). Participants were genotyped for SNP rs2910164 in miR-146a. Analyses investigating association were adjusted for relevant clinical covariates including age, sex, DM duration, Hb_{A1c} and hypertension.

Results: A significant association was found between the C allele of rs2910164 and DN in the T1DM group (OR, 1.93; CI, 1.23-3.03; P=0.004), but no association found in the T2DM group (OR, 1.05; CI, 0.83-1.32; P=0.691). In the subset of T2DM patients, the C allele was specifically associated with DME (OR, 1.25; CI, 1.03-1.53; P=0.025). No association with DME was found in the T1DM group (OR, 0.87; CI, 0.54-1.42); P=0.583), or with PDR for either type of DM.

Conclusions: Rs2910164 is significantly associated with microvascular complications DN in patients with T1DM and DME in patients with T2DM.

Keywords:

Diabetic retinopathy; diabetic macular edema; diabetic nephropathy; microRNA; microRNA-146a; genetics.

Introduction:

The incidence of diabetes mellitus (DM) is increasing worldwide [1, 2]. With this rise comes an increase in patients with microvascular complications including diabetic retinopathy (DR) and diabetic nephropathy (DN). Multiple mechanisms have been postulated to contribute to the pathogenesis of the microvascular complications of DM, many of which involve activation of inflammation associated with hyperglycemia-induced elevation of oxidative stress [3]. Nuclear factor-kappaB (NF-κB), a nuclear transcription factor, regulates multiple gene pathways involved in inflammation, immune response and apoptosis, and has been implicated in the development of DM complications [3, 4].

Micro-RNAs (miRs) are short, non-coding RNA molecules, which regulate mRNA stability and translation by binding to their target mRNAs [5]. Following transcription, pre-miRs assume a hairpin structure which is then cleaved to form two mature miR duplex strands, each given a suffix of either -5p or -3p [5, 6]. miR-146a-5p is an NF-kB responsive miRNA. By targeting negative regulators of angiogenic signalling pathways, miRs play an important role in modulating endothelial cell proliferation and vascular development in response to both physiological and pathological events [7].

MiR-146a is located on chromosome 5q33, and in common with most microRNAs, has multiple gene targets [8]. It plays an important role in the innate immune response and is associated with autoimmune diseases [9] and inflammation [10]. Post-transcriptional gene silencing by miR-146a inhibits the NF-κB inflammatory cascade via a number of known pathways [11, 12]. NF-kB responsive miRNAs, including miR-146a, are upregulated in retinal endothelial cells of diabetic patients [13]. NF-κB functions as an important regulator of endothelial cell apoptosis by activating pro-apoptotic factors in response to cellular stressors such as hyperglycemia [4, 14]. Mir-146a also targets fibronectin, an extracellular matrix (ECM) protein deposited in end organs (including retina, kidney, heart) affected by DM [15].

Rs2910164 is a common single nucleotide polymorphism (SNP) located within the seed sequence of miR-146a-3p, and is predicted to lead to perturbation of pairing of the hairpin strands [16]. Presence of the minor allele (C) reduces the processing efficacy of pre-miR-146a into the mature miR-146a form, resulting in an overall reduction in mature miR-146a [16]. Given the function of miR-146a as an inhibitor of NF-κB-activated inflammation and fibronectin gene transcription, changes in miR-146a levels secondary to the rs2910164 SNP, could plausibly have functional implications in the pathogenesis of diabetic microvascular complications. This study aimed to investigate whether rs2910164 is associated with the diabetic complications, sight threatening DR (STDR) and diabetic nephropathy (DN), in two large and well-characterised cohorts of Caucasian patients with type 1 (T1DM) and type 2 (T2DM) diabetes.

Methods:

Participant recruitment for this study occurred at ophthalmology, endocrine, and renal clinics from multiple sites within Australia and the United Kingdom. The following centres were involved, with study approval by their respective Human Research Ethics Committees HREC: Flinders Medical Centre (Southern Adelaide Clinical HREC), The Repatriation General Hospital (Southern Adelaide Clinical HREC), The Royal Adelaide Hospital (Royal Adelaide Hospital HREC), The Queen Elizabeth Hospital (The Queen Elizabeth Hospital HREC), The Royal Melbourne Hospital (Royal Melbourne Hospital HREC), Royal Victorian Eye and Ear Hospital (Royal Victorian Eye and Ear Hospital HREC), St Vincent's Hospital, Melbourne (St Vincent's Hospital HREC), Sydney Eye Hospital (South Eastern Sydney Illawarra HREC), and Canberra Hospital (ACT Health HREC) in Australia; and The National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom (The NHS Health Research Authority in London). Written informed consent was obtained from each participant. The project conformed to the tenets of the Declaration of Helsinki.

Adult patients (>18 years of age) were eligible for participation in this study if they had a history of either T1DM, or T2DM treated with either oral hypoglycemic agents and/or insulin therapy, for at least five years. Recruitment methodology and baseline characteristics of cases and controls have been reported [17]. Since this report, an additional 1230 diabetic patients (639 with sight-threatening DR (STDR)) have been recruited with similar baseline characteristics. All patients completed a questionnaire (including social, demographic and medical history), underwent ophthalmic examination to determine DR grading (using the modified Early Treatment Diabetic Retinopathy Study criteria grading system [18]), and provided a venous blood sample. Hb_{A1c}, serum lipids, serum creatinine and urinary albumin were obtained from patient records.

Cases for nephropathy analyses were defined as those who were on dialysis, or who had received a renal transplant for DN, or, those with a 24 hour urine albumin of at least 30mg/d or eGFR <60 mls/min, according to their most recent renal function results. Those with urinary albumin less than 30mg/d, eGFR>60 mls/min, and no history of dialysis or transplant for DN made up the control group for nephropathy analyses. DR grading was used to classify patients into case and control groups for retinopathy analyses. Cases with STDR, defined as a worst ever eye grading of severe non-proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), or diabetic macular edema (DME), in at least one eye, were compared against diabetic controls whose retinopathy grading had never been worse than minimal NPDR, with no history of DME in either eye. Case/control analyses for each phenotype examined were performed separately for T1DM and T2DM groups (ie. T1DM cases were compared against T1DM controls etc.).

DNA was extracted from whole blood using QIAamp Blood DNA Maxi Kits (Qiagen). MiR-146a SNP rs2910164 was genotyped in all individuals using iPLEX Gold chemistry on an autoflex Mass Spectrometer (Sequenom, San Diego, CA). This specific SNP was chosen for a number of reasons. Firstly, this SNP is the only common variant located within the miRNA gene MIR146A and has been shown to alter the processing of the miRNA, with the C allele resulting in less mature miRNA. There are several other very rare variants mapping to this gene however this study would not have power to detect any association and there is currently no evidence of their functionality. Rs2910164 has also been studied previously in relation to other diseases.

Rs2910164 was tested for Hardy-Weinberg equilibrium (HWE) in case and control groups. Statistical Package for Social Sciences (SPSS) version 20.0 (IBM SPSS Statistics for Windows Version 20.0; IBM Corp., Armonk, NY, USA) was used for basic descriptive statistics to determine baseline clinical characteristics of the cases and controls. The Mann-Whitney U test was used for continuous variables whilst χ^2 test was used for dichotomous variables. PLINK (version 1.06) was used to test for association of rs2910164 with cases versus controls. Univariate analysis was performed with the χ^2 test for dominant and recessive models and the Cochran-Armitage test for the additive model. Multivariate analysis, adjusting for age, sex, duration of DM, Hb_{A1c}, hypertension, nephropathy and retinopathy was performed with binary logistic regression analysis. Results are presented for additive, dominant and recessive models. Where there was *a priori* evidence of an association with STDR, testing for association for STDR sub-phenotypes (PDR and DME) versus controls was performed. Adjustment for the 2 hypotheses tested (DN and STDR), resulted in a P-value of less than 0.025 required for significance. For the subgroup of patients with T2DM, this study was 80% powered to detect a minimal odds ratio (OR) of 1.3 (for dominant and additive models), with the significance level set at 0.025 (to account for multiple comparisons)[19]. The recessive model requires a larger OR to be detectable in this study. For patients with T1DM, and a significance level of 0.025, this study was powered (80%) to detect an OR of 1.3 for an additive model, for DN and STDR analyses only [19].

Results:

Participants (n= 2948) with T1DM (n=733) or T2DM (n=2215) were successfully genotyped. Genotype frequencies were in HWE for both case (p=0.999) and control groups (p=0.999). Clinical characteristics of the group, for T1 and T2 DM types are presented in Table 1.

Diabetic Nephropathy

A total of 890 participants had DN, including 168 participants with T1DM, and 722 participants with T2DM (Table 1). This included 38 participants on dialysis and 13 participants who had received a kidney transplant for DN. The remaining 2058 participants had no DN (565 with T1DM and 1493 with T2DM). Clinical characteristics of cases and controls are presented in supplementary table 1.

Genotype frequencies for DN controls and cases for T1DM or T2DM patients are shown in table 2. A significant association between genotypes containing the C allele and DN in the T1DM cohort was found after adjustment for clinical covariates for additive (OR, 1.63; CI, 1.13-2.35; P=0.008) and dominant (OR, 1.93; CI, 1.23-3.03; P=0.004) models (Table 3). No significant difference was found in the T2DM cohort.

Diabetic Retinopathy

One thousand, one hundred and fifty three participants were classified as DR controls (258 with T1DM and 895 with T2DM), and 1315 participants satisfied criteria for STDR (330 with T1DM and 985 with T2DM) (Table 1). The remaining 480 participants had mild or moderate NPDR and were not included in case control retinopathy analyses. Of those with STDR, 731 participants had PDR (with 251 T1DM and 480 with T2DM) and 1026 had DME (170 T1DM and 856 with T2DM). Clinical characteristics of cases and controls are presented in supplementary table 2.

Genotype frequencies for controls and cases (combined and stratified into PDR and DME groups) are shown in Table 2. In the T2DM group, univariate analysis with a dominant model showed a trend towards an association with STDR (P=0.059). When the composite phenotype of STDR was then analysed by it's sub-phenotypes PDR and DME, a stronger association was found between those with genotypes containing the minor allele (dominant model) and PDR (P=0.037), as well as DME (P=0.019). In an additive model analysis, the C allele was also found to be associated with DME (P=0.043). The significant associations with DME were maintained after adjustment for age, sex, duration of DM, Hb_{A1c}, hypertension and nephropathy (additive model: OR, 1.25; CI, 1.03-1.53; P=0.025; dominant model: OR, 1.29; CI, 1.01-1.65; P=0.044), and in the additive model, the association with DME survived adjustment for multiple testing. After multivariate analysis, the association with PDR was of only borderline significance (P=0.051). No significant association was found between rs2910164 and STDR in the smaller T1DM subgroup alone.

Discussion:

This is the first study to investigate the common miR-146a SNP at rs2910164 and its relationship with diabetic microvascular complications. In our study, the C allele was found to be significantly associated with DN in patients with T1DM after multivariate analysis. The lack of association with univariate analysis can be explained by the influence of confounding variables. The effects of these variables are adjusted for with the use of multivariate analysis, which is particularly important in a complex disease, influenced by multiple environmental factors such as diabetes. The frequency of the C allele was also found to be greater in patients with STDR than in diabetic controls in those with T2DM. Sub-type analyses showing similar effect sizes in both PDR and DME phenotypic subgroups suggest that this SNP may increase susceptibility to retinal damage via a pathway involved in both angiogenesis and blood retinal barrier breakdown. The functionally important C allele specifically confers significant risk for developing DME in patients with T2DM. PDR and DME sub-group analyses in the T1DM group were underpowered to detect an association.

The literature relating to rs2910164 presents contrasting results regarding the risk allele across varying diseases and ethnicities. The CC genotype has been associated with increased risk for a number of diseases including coronary artery disease in Indian males [20]. On the other hand, the C allele and CC genotype have been found to be protective against the development of cardiovascular diabetic autonomic neuropathy in a Caucasian population [21]. The opposite (protective) effect of the C allele found by Ciccaci et al. compared with the current study may be due to the study size (only 11 cases with cardiovascular diabetic autonomic neuropathy compared with 100 controls). Alternatively, there may in fact be different genetic risks for various diabetic complications.

It is well accepted that altered expression of miR-146a in the hyperglycemic (and pro-inflammatory) environment negatively regulates NF-κB expression via a negative feedback loop [13, 15]. Studies have consistently shown that NF-κB activation correlates with the apoptosis of pericytes in the presence of sustained hyperglycaemia, and precedes histopathological changes characteristic of diabetic retinopathy such as pericyte ghosts [4, 22]. Furthermore, high glucose induced bovine retinal endothelial cell and pericyte apoptosis can be prevented by directly inhibiting NF-κB [4]. Jazdzewski et al. correlated miR-146a genotypes with mature miR-146a levels. They showed a 1.8 fold decrease in mature miR-146a from the C allele compared to the G allele at rs2910164 [16]. They also found that the C genotype results in less inhibition of *IRAK-1* and *TRAF6* than the G genotype, confirming a direct functional effect of rs2910164 on miR-146a target genes [16]. These findings imply that the C allele has impaired ability to dampen NF-κB mediated inflammation, which could be of relevance to the pathological role of the C genotype in the development of DN and STDR suggested by the current study.

Feng et al. investigated the role of miR-146a on fibronectin expression, a second target of miR-146a [15]. They found that reduced miR-146a expression directly increases synthesis of fibronectin, an ECM glycoprotein found in microvascular endothelial cells. Increased fibronectin synthesis was seen in a number of end organs including retina, kidney and heart in a STZ rat model [15]. Enhanced production of ECM proteins, including fibronectin, is characteristic of DM microvascular pathology. The deposition of excess fibronectin in human retina has been shown to bind endothelial cell transmembrane receptors, integrins (also located on chromosome 5q), which play a significant role in vascular permeability and ocular neovascularisation [23]. Increasing the availability of miR-146a with intravitreal injection of a miR-146a mimic was able to block DM-induced fibronectin upregulation in a diabetic rat model [15]. Further investigation of this, and other miR-146a targets is warranted to gain a better understanding of how miR-146a could be manipulated for therapeutic use.

There have been a number of studies investigating the effects of hyperglycemia on miR-146a function and expression. Human kidney tissue from patients with DN (stage IV-V) showed a 4.87 fold upregulation of Mir-146a compared with non-diabetic kidney tissue, with highest expression in glomerular endothelial cells (ECs), mesangial areas and tubular sections of the diabetic kidney [24]. The increase in Mir-146a expression in kidney tissue has been reported to correlate with progression of DN in Streptozotocin (STZ) rats [24]. Similar increases in miR146a expression have been found in retinal endothelial cells of rats 2-3 months after STZ-induced DM [13]. It is possible that Mir-146a is upregulated in end organ tissues in an attempt to protect against the increase in hyperglycaemia induced inflammation that occurs during the progression of microvascular complications of diabetes. It would be interesting to explore the rate of progression of end organ damage in those with and without the Mir-146a risk genotype reported in this study.

The predominant limitation of this study was the lack of power for PDR and DME sub-analyses in the smaller T1DM group. This may explain the negative findings in the T1DM analysis for these phenotypes. The small to moderate effect size assessed in this study (OR=1.3) was based on the fact that DM complications are likely to be a result of complex gene-environment interactions, and the impact of a single

SNP is therefore likely to be small. It may be that an even smaller effect size needs to be considered for the impact of this SNP, and that the T2DM cohort was underpowered to detect an effect of this size. Thus, it is possible that the DME association in T2DM patients found in the current study (of borderline significance and small OR of 1.25) is in fact a false positive result. Further studies with a larger cohort size are required to more accurately explore these specific phenotypes with relation to miR-146a SNPs.

Given that T1DM and T2DM are distinct diseases with differing aetiologies, association of SNPs specific to one type of DM is also a plausible explanation. The development of DN and DR are influenced by environmental factors that may be more likely to occur in the context of a specific type of DM. For example, it is known that DN most commonly develops over the first 10 to 15 years of DM in susceptible patients with T1DM, but in patients with T2DM, the timing is less clearly defined[25]. Mean duration of diabetes in our studies was 23 years for those with T1DM and 16 years for those with T2DM, covering the highest risk timeframe for those with T1DM but perhaps not those with T2DM. This may be another explanation for the significant association found in patients with T1DM but not with T2DM in the current study. Type and duration of treatment of T2DM may also be related to the development of DN and was not considered as a potential confounding factor in our analysis, as this study was not designed to adequately collect longitudinal data on diabetes treatment. Finally, it may be useful to consider this SNP's association with end-stage renal disease (ESRD) rather than with DN as a whole, as this study was not powered to explore whether rs2910164 is associated with onset of DN, or progression to ESRD.

Conclusion:

This study reports for the first time that rs2910164 is associated with DN in those with T1DM, and STDR and its subgroup DME in Caucasian patients with T2DM. There is evidence for a direct link between the CC genotype, decreased levels of mature miR-146a and downstream failure of regulation of NF-κB mediated inflammation. Predictive studies measuring mir-146a levels or identifying those with the rs2910164 risk genotype have potential to contribute to the identification of DM patients at high risk of complications. Furthermore, manipulation of MiR-146a has therapeutic potential for the treatment or prevention of these diabetic microvascular complications.

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Statement of Human and Animal Rights: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Statement of Informed Consent: Informed consent was obtained from all patients for being included in the study.

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Figures and tables:

Table 1: Demographic and clinical characteristics of participants by type of diabetes.

Clinical characteristics	T1DM n=738	T2DM n=2230
Female, n (%)	358 (48.5)	1022 (45.8)
Age, years, mean (SD)	43.6 (16.4)	66.2 (11.4)
Duration of DM, years, mean (SD)	23.0 (13.7)	16 (9.2)
Insulin treatment, n (%)	-	1105 (49.6)
Hb_{A1c}, % (mmol/mol), mean (SD)	8.3 (67) (1.7)	7.9 (63) (1.67)
BMI, mean (SD)	27.1 (5.4)	31.7 (6.6)
Hyperlipidemia, n (%)	334 (45.2)	1536 (68.9)
Hypertension, n (%)	333 (45.1)	1679 (75.3)
Smoker, n (%)	325 (44.0)	1050 (47.1)
Nephropathy, n (%)	168 (22.8)	724 (32.5)
Dialysis, n (%)	6 (0.8)	32 (1.4)
Transplant, n (%)	3 (0.4)	10 (0.4)
STDR, n (%)	331 (44.9)	991 (44.4)
PDR, n (%)	252 (34.1)	482 (21.6)
DME, n (%)	170 (23.0)	863 (38.7)

Abbreviations: BMI, body mass index; STDR, sight threatening diabetic retinopathy; Hb_{A1c}, hemoglobin A_{1C}; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; SD standard deviation. Nephropathy defined as those with 24 hour urine albumin ≥30mg/d, or eGFR <60 mls/min, or a history of dialysis or transplant for DN. STDR defined as severe NPDR or PDR, and/or DME. Controls defined as no DR or minimal NPDR. Patients with DME can have co-existing PDR or NPDR.

Table 2: Genotype frequencies, shown as n (%) for Controls and Cases of each phenotype tested, for each type of DM.

Phenotype	Genotype	Controls	Cases		
T1DM Nephropathy		**(n=565)**		**(n=168)**	
	CC	31 (5.5)		12 (7.1)	
	GC	199 (35.2)		68 (40.5)	
	GG	335 (59.3)		88 (52.4)	
Retinopathy		**(n=258)**	**STDR (n=330)**	**PDR (n=251)**	**DME (n=170)**
	CC	13 (5.0)	21 (6.4)	14 (5.6)	9 (5.3)
	GC	89 (34.5)	119 (36.0)	101 (40.2)	52 (30.6)
	GG	156 (60.5)	190 (57.6)	136 (54.2)	109 (64.1)
T2DM Nephropathy		**(n=1493)**		**(n=722)**	
	CC	104 (7.0)		46 (6.4)	
	GC	551 (36.9)		283 (39.2)	
	GG	838 (56.1)		393 (54.4)	
Retinopathy		**(n=895)**	**STDR (n=985)**	**PDR (n=480)**	**DME (n=856)**
	CC	55 (6.1)	70 (7.1)	31 (6.5)	69 (8.1)
	GC	327 (36.5)	393 (39.9)	202 (42.1)	344 (40.2)
	GG	513 (57.3)	522 (53.0)	247 (51.5)	443 (51.8)

Abbreviations: T1DM, type 1 diabetes mellitus; T2DM type 2 diabetes mellitus; DN, diabetic nephropathy; STDR, sight threatening diabetic retinopathy; PDR, proliferative diabetic retinopathy; DME, diabetic macular edema.
DN cases defined as those with 24 hour urine albumin ≥30mg/d, or eGFR <60mls/min, or a history of dialysis or transplant for DN. DN controls defined as those with 24 hour urinary albumin less than 30mg/d, eGFR>60 mls/min, and no history of dialysis or transplant for DN. STDR defined as severe NPDR or PDR, and/or DME. DR controls defined as no DR or minimal NPDR. Patients with DME can have co-existing PDR or NPDR.

Table 3: Association of *Mir146a* SNP rs2910164 (C allele) with DN, for T1DM and T2DM groups individually. Univariate and multivariate analyses, for additive, dominant and recessive models are presented.

	DN Cases versus Controls			
	T1DM		T2DM	
Univariate	**CATT or X^2**	**P value**	**CATT or X^2**	**P value**
Additive	Z=2.66	0.264	Z=1.19	0.553
Dominant	X^2=2.53	0.111	X^2=0.57	0.451
Recessive	X^2=0.64	0.422	X^2=0.27	0.602
Multivariate	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Additive[a]	1.63 (1.13-2.35)	**0.008***	1.03 (0.86-1.25)	0.734
Dominant[a]	1.93 (1.23-3.03)	**0.004***	1.05 (0.83-1.32)	0.691
Recessive[a]	1.42 (0.56-3.62)	0.458	1.01 (0.63-1.62)	0.962

Abbreviations: T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; OR (95%CI), odds ratio with 95% confidence interval; CATT, Cochran-Armitage Trend Test. DN cases defined as those with 24 hour urine albumin ≥30mg/d, or eGFR <60 mls/min, or a history of dialysis or transplant for DN. Controls defined as those with 24 hour urinary albumin less than 30mg/d, eGFR>60 mls/min, and no history of dialysis or transplant for DN. [a]Adjusted for age, sex, duration DM, Hb_{A1c}, hypertension, retinopathy. P<0.05 in bold. *Significant association with adjustment for multiple testing (P < 0.025).

Table 4: Association of *Mir146a* SNP rs2910164 (C allele) with STDR and its subtypes PDR and DME, for T1DM and T2DM groups individually. Univariate and multivariate analyses, for additive, dominant and recessive models are presented.

	STDR Cases Vs Controls		**PDR Cases Vs Controls**		**DME Cases Vs Controls**	
T1DM						
Univariate	**CATT or X^2**	**P value**	**CATT or X^2**	**P value**	**CATT or X^2**	**P value**
Additive	Z=0.75	0.689	Z=2.07	0.355	Z=0.71	0.701
Dominant	X^2=0.50	0.480	X^2=2.05	0.152	X^2=0.58	0.446
Recessive	X^2=0.47	0.495	X^2=0.07	0.786	X^2=0.01	0.907
Multivariate	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Additive[a]	1.23 (0.84-1.79)	0.293	1.24 (0.81-1.89)	0.324	0.87 (0.54-1.42)	0.583
Dominant[a]	1.15 (0.72-1.85)	0.562	1.25 (0.74-2.09)	0.409	0.84 (0.46-1.52)	0.558
Recessive[a]	1.99 (0.78-5.04)	0.148	1.54 (0.53-4.46)	0.430	0.89 (0.25-3.16)	0.853
T2DM						
Univariate	**CATT or X^2**	**P value**	**CATT or X^2**	**P value**	**CATT or X^2**	**P value**
Additive	Z=3.63	0.163	Z=4.49	0.106	Z=6.27	**0.043**
Dominant	X^2=3.54	0.059	4.34	**0.037**	5.47	**0.019***
Recessive	X^2=0.70	0.403	X^2=0.05	0.819	X^2=2.44	0.118
Multivariate	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Additive[a]	1.17 (0.97-1.43)	0.107	1.22 (0.94-1.57)	0.137	1.25 (1.03-1.53)	**0.025***
Dominant[a]	1.21 (0.95-1.54)	0.127	1.37 (1.00-1.87)	0.051	1.29 (1.01-1.65)	**0.044**
Recessive[a]	1.27 (0.78-2.07)	0.338	0.90 (0.45-1.78)	0.758	1.46 (0.90-2.37)	0.122

Abbreviations: DM, diabetes mellitus; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; OR (95%CI), odds ratio with 95% confidence interval; CATT, Cochran-Armitage Trend Test. Controls defined as no DR or minimal non-proliferative diabetic retinopathy (NPDR). STDR (sight threatening diabetic retinopathy) defined as severe NPDR or PDR, and/or DME. PDR, proliferative diabetic retinopathy; DME, diabetic macular edema. [a]Adjusted for age, sex, duration DM, Hb_{A1c}, hypertension, nephropathy. P<0.05 in bold. *Significant association with adjustment for multiple testing (P < 0.025).